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TITLE: The Role of the Interferon-Gamma-Jak/STAT Pathway in Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR: Stanley Louis Bridges, Jr., MD, PhD

CONTRACTING ORGANIZATION: University of Alabama at Birmingham
                                           Birmingham, AL 35294-0001

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                             Fort Detrick, Maryland 21702-5012

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
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The Role of the Interferon-Gamma-Jak/STAT Pathway in Rheumatoid Arthritis

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6. AUTHOR(S)
Stanley Louis Bridges, Jr., MD, PhD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Type I (IFN-α) and type II (IFN-γ) interferons are important mediators of autoimmunity. Our group recently showed a strong association of IFN-γ receptor 1 (*Ifngr1*) expression and of IFN-γ receptor 2 (*Ifngr2*) expression in peripheral blood mononuclear cells (PBMC) with the presence of RA and its radiographic severity, respectively (*Arthritis Rheumatol.* 2015 67:1165). IL-2 has essential regulatory function in inflammatory diseases and is considered as a potential therapy for autoimmune disease. We tested the hypothesis that RA is associated with alterations in IFN-γ and IL-2 STAT signaling within certain subsets of PBMCs. We used a high-definition phospho-flow approach to evaluate the activation of STAT1, STAT3 and STAT5 after IFN-γ or IL-2 stimulation. We analyzed PBMCs from 37 RA patients and 12 healthy controls (HC) for activation of STATs in specific CD4 and CD8 T cells subpopulations, B cells and monocytes. We found that IFN-γ induced STAT1 activation was significantly greater in RA naïve, central memory, Tfh and Treg subsets of CD4+ T cell populations compared to HC ($p<0.05$). IL-2 very efficiently activated STAT5 in all T and B cell populations in RA and HC. The activation of STAT5 in RA was significantly greater than HC in only one population: effector memory CD4 T cells ($p<0.01$). Our studies revealed the presence of a STAT5 phosphatase in RA T cell subsets that likely counteracts IL-2 regulator activity and contribute to the pathogenesis of RA.

15. SUBJECT TERMS
Rheumatoid arthritis; Autoimmunity; T lymphocyte subsets; Cell Signaling; Interferon-gamma; STAT1; STAT3; STAT5: Interleukin-2

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
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Unclassified	Unclassified	Unclassified			

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Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

This project addresses the hypothesis that elevated and/or altered IFN-γ signaling within selective subsets of mononuclear cells promotes disease severity in RA. This study developed from our novel observation that in peripheral blood the expression levels of interferon gamma receptor 1 (IFNGR1) is associated with RA and the expression levels of IFNGR2 correlates significantly with the degree of radiographic damage in RA patients. The aims of this proposal are: (1) To identify the specific circulating cell type in which IFNGR expression is elevated in RA. Using a combination of molecular biological and immunological approaches, we will analyze the expression levels of IFNGR1 and IFNGR2 in monocytes, naïve and memory B cell populations, naïve and memory T cell populations including T-follicular helper cells, Treg cells and T helper effector subpopulations (Th1, Th17 and Th17/1). (2) To determine the outcome of IFNGR signals by assaying the activation of IFN-γ induced STAT1 and changes in activation of STAT3 and STAT5 in RA versus healthy controls, at basal level and following stimulation with cytokines such as IL-2, IL6 etc. (3) To determine the molecular mechanism and outcome of attenuated IL-2 induced activation of STAT5 in specific subpopulations of T cells in RA. The information to be gained can potentially help to identify new cell signaling targets, perhaps cell-type specific, for RA and other autoimmune diseases, and perhaps malignancies. This in turn may help to develop new drugs that are more targeted, either to particular cell types or patients in whom these cell types are most important to the disease. Ultimately, this may lead to more effective, and safer drugs with fewer adverse effects.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Rheumatoid arthritis; Autoimmunity; T lymphocyte subsets; Cell Signaling; Interferon-gamma; STAT1; STAT3; STAT5: Interleukin-2

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1 (specified in proposal)	Timeline	Status
Major Task 1 - To identify the circulating cell types in which IFNGR expression is upregulated in RA and determine how it relates to disease activity.	Months	**Completed, % complete, or Future Work**
Subtask 1 – To recruit 250 participants for Major Tasks 1, 2, and 3. This includes 150 with RA (50 each with low disease activity/remission; moderate disease activity; high disease activity); 50 with multiple sclerosis; 50 healthy controls. Collect data,	Begin Month 4 (after IRB approval); end Month 27	Recruitment is approximately 50% completed, with 127 patients enrolled to date.

including disease activity, medications, demographics, etc.		
Subtask 2 – Perform FACS and quantitative real-time PCR (qRT-PCR) to measure IFNGR1 and IFNGR2 expression in multiple T cell and B cell populations and monocytes.	Begin Month 4 and proceed in batches; end Month 27	Conditions for sorting for populations have been established. We will complete task by end of Month 27.
Subtask 3 - Assess IFN-γ receptor protein levels in cell subsets (including Th1, Th17, Th17/1, etc.) in RA with different disease activity (remission/low; moderate; high), MS, and controls.	Begin Month 4 and proceed in batches; end Month 27	These studies have been initiated and on target.
Subtask 4 - We will compare results among patients with RA with different disease activity (remission/low; moderate; high), MS, and controls.	Begin Month 7 and proceed throughout the funding period	Comparison of results will be performed when adequate numbers of each disease group/severity/control are assayed. We anticipate this will begin Month 13.
Milestone(s) Achieved		
Local IRB Approval	3	6-14-17
HRPO Approval	6	HRPO Log Number A-19648 - approved on August 1, 2016
Present results at scientific meetings	18, 24	Future
Publish results in scientific journals	24, 30	Future
Specific Aim 2 (specified in proposal)		
Major Task 2 - To determine the effect of upregulated IFNGR expression on IFN-γ-induced activation of STAT1, STAT3, and STAT5 signaling in peripheral blood cell subsets in RA.		
Subtask 1 - Compare the level of activation of STATs (as assessed by the degree of phosphorylation) in peripheral blood naïve and memory CD4+ T cells, Th effector populations, Treg, naïve and memory B cells, and monocytes at baseline and following stimulation with IFN-γ in RA (n=150) using phospho-flow cytometry.	Begin Month 4 and proceed in batches; end Month 27	This task is ongoing. We have completed initial analysis of peripheral blood populations form 37 RA patients, 4 MS patients and 12 HC.
Subtask 2 - Determine if altered STAT1 (or STAT3 or STAT5) activation leads to differences in nuclear localization of STAT1 (or STAT3 or STAT5) followed by changes in cellular morphology in different mononuclear	Begin Month 4 and proceed in batches; end Month 27	This task will be initiated beginning month 14. However, we will complete the task by month 27. Two new

subpopulations using quantitative image analysis and flow cytometry (Imagestream).		graduate students (see below) joined the project and developing expertise on other technologies related to the project was given precedence.
Subtask 3 - Determine if IFN-γ signals alter the ability of other cytokines (IL-2, GM-CSF, IL-6, IL-23) to activate their respective STATs.	Begin Month 4 and proceed in batches; end Month 27	This task has been initiated. Current results are preliminary.
Present results at scientific meetings	18, 24	Future
Publish results in scientific journals	24, 30	Future
Specific Aim 3 (specified in proposal)		
Major Task 3 - To determine the molecular mechanism and outcome of attenuated IL-2 induced activation of STAT5 in specific subpopulations of T cells in RA.		
Subtask 1 – Determine whether altered IL-2 mediated activation of STAT5 in subpopulations of T cells in RA contributes to disease pathogenesis.	Begin Month 4 and proceed in batches; end Month 27	We have so far analyzed peripheral blood from 17 RA patients and 10 HC. Progress is currently on target.
Subtask 2 – Determine the outcome of attenuated IL-2 mediated activation of STAT5 on Th effector cell and regulatory cell expansion and function.	Begin Month 4 and proceed in batches; end Month 27	This subtask has not been initiated and will be initiated beginning month 14. The reasons are described in Major Task 3 – subtask 2 (see above).
Present results at scientific meetings	18, 24	Future
Publish results in scientific journals	24, 30	Future

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities
I. *Collection of Blood samples from RA, MS (multiple sclerosis) and healthy controls (HC).*
a. Target: 150 RA patients (50 remission/low disease, 50 moderate disease, 50 high disease); collected 91 RA patients.

b. Target: 50 newly diagnosed treatment naïve MS patients; collected peripheral blood cells from 22 MS patients. Three MS Neurologists, Drs., Bashir, Rinker and Meador provide the heparinized peripheral blood from which peripheral blood mononuclear cells are isolated and cryopreserved.
c. Target: 50 HC; collected 14 HC.

II. *Recruitment and training of graduate students*:
Two new graduate students, Mr. Vishal Sharma (Ph.D. Immunology program) and Mr. Brandon Pope (MD/Ph.D. program) were recruited in March 2017 for the studies in this proposal. They have developed proficiency in almost all of the tools necessary for this project.

2) Specific objectives.
Aim 1. To identify the circulating cell types in which IFNGR expression is upregulated in RA and determine how it relates to disease activity.
Mr. Vishal Sharma and Mr. Brandon Pope have developed the expertise to sort by flow cytometry the T cell populations, the B cell populations and monocytes. Beginning Month 11, we will begin interrogating these cell populations for the expression of IFNGR1 and IFNGR2 by quantitative PCR and flow cytometry. Our current approach is further refined from originally described in the proposal. We have now the ability to identify and sort specific T cell and B cell populations with greater specificity.

Aim 2. To determine the effect of upregulated IFNGR expression on IFN-γ-induced activation of STAT1, STAT3, and STAT5 signaling in peripheral blood cell subsets in RA.
The greatest progress has been made in objectives of Aim 2. We have analyzed the activation of STAT1 induced by IFN-γ in 37 RA, 4 MS and 12 HC. We now interrogate IFN-γ induced SAT1 activation along with the activation of STAT3 and STAT5 over a range of stimulation time-points. This approach is feasible as each stimulation condition requires only 10^5 cells, which represents five-fold less than that used in our preliminary studies. We are now comparing both the extent of STAT activation in various T cell populations as well as duration of response between RA patients of different disease severity, treatment naïve MS patients and HC.

Aim 3. To determine the molecular mechanism and outcome of attenuated IL-2 induced activation of STAT5 in specific subpopulations of T cells in RA.
We have analyzed IL-2 induced STAT5 activation in 17 RA patients and 10 HC. We initially observed enhanced IL-2 induced STAT5 activation only in RA effector memory CD4 T cells compared to HC. The expected result was enhanced IL-2 induced activation of STAT5 in RA Treg cells and Tfh cells, as regulatory feedback to disease. We therefore hypothesized that a phosphatase in RA T cells dephosphorylates active STAT5 (phospho-STAT5). To test for this, PBMC were pretreated with phosphatase inhibitors before stimulation with IL-2. Remarkably, our results reveal the existence of a phosphatase that selectively acts on p-STAT5 in RA T cells.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

Aim 2. We interrogated IFN-γ induced activation of STAT1 (pY701), STAT3 (pY705) and STAT5 (pY694) in peripheral blood T cell subpopulations from healthy controls, RA patients of

varying severity and newly diagnosed treatment naïve MS patients using phospho-flow cytometry. In all CD4 and CD8 T cell sub populations, we observed no difference in basal levels of pSTAT1, pSTAT3 or pSTAT5 between HC, RA patients and treatment naïve MS patients (Fig 1 and data not shown). In RA, IFN-γ induced activation of STAT1 was greatest in remission (CDAI ≤2.8), and lowest in individuals with moderate disease (Fig. 1). In fact, in CD4 naïve and central memory T cells, we observe a trend towards increased IFNγ-induced STAT1 activation with decrease in disease severity within (Fig. 1). Such differences were not observed in CD8 T cell populations (data not shown). Remarkably, MS patients had the highest IFN-γ induced STAT1 activation, proximal to that observed in RA patients in remission (Fig. 1). The enhanced IFN-γ induced STAT1 activation with decrease in disease severity might suggest a protective role for IFN-γ. This will be explored in the coming year.
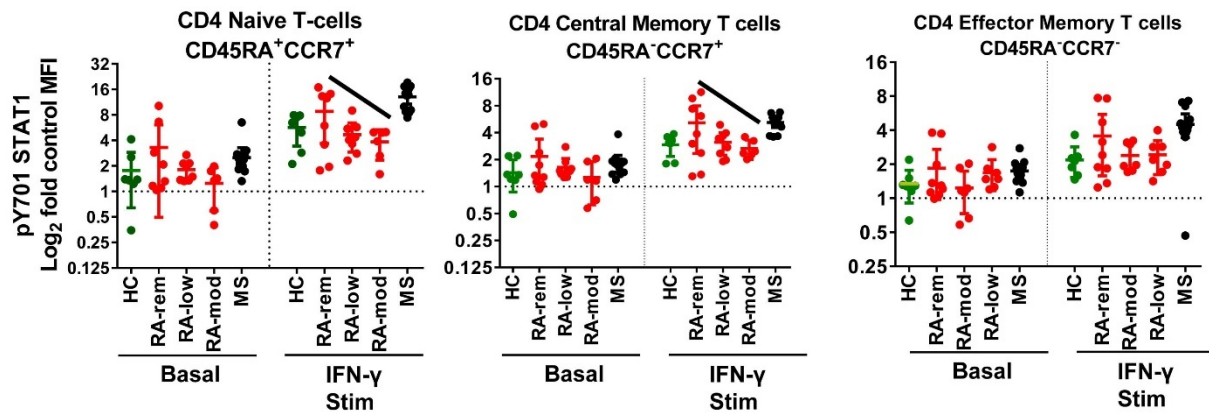

Fig. 1. IFN-γ induced STAT1 activation (pY701-STAT1) in RA increases with disease severity. pY701-STAT1 at basal and following IFN-γ (50 ng/ml) stimulation in naïve (CD45RA+CCR7+), central memory (CD45RA-CCR7+) and effector memory (CD45RA-CCR7-) CD4 T cells from healthy controls (HC), remission RA (CDAI≤2.8), low RA (CDAI >2.8≤10), moderate RA (CDAI>10<22) and treatment naïve newly diagnosed MS. Each dot represents an individual patient or control. Data is mean ± 95% confidence interval.

Aim 3. We evaluated the levels of pSTAT5-Y694 (pSTAT5) in CD4 and CD8 T cell populations following stimulation with IL-2. We found that the activation of STAT5 by IL-2 in effector memory was significantly greater in RA than in HC (Fig. 2). IL-2 induced pSTAT5 in naïve and central memory CD4 T cells was higher in RA compared HC, but the difference was not statistically significant. In all other CD4 T cell populations, including Treg cells, we observed no difference in the activation of IL-2 induced STAT5 between RA and HC (data not shown). We also observed no difference in IL-2-induced activation of STAT5 between RA and HC in all CD8 T cell populations (data not shown).

The data above suggests that IL-2 dependent regulation of IFN-γ is normal in RA. However, considering that RA is characterized by chronic inflammation, one would predict IL-2 dependent activation of STAT5 to be greater in RA than HC. We therefore hypothesized that optimal STAT5 activation was dampened in RA. We found no difference in levels of total STAT5 between RA (data not shown). Phosphatases play a key role in regulating STAT activation. We therefore interrogated if addition of phenylarsine oxide (PAO), a broadly active phosphatase inhibitor, alters the activation of STAT5 by IL-2. We observed that in several CD4

T cell populations, the addition of PAO during IL-2 stimulation greatly enhanced the activation of STAT5 (Fig. 3). The enhanced activation of STAT5 was more pronounced in RA than in HC.
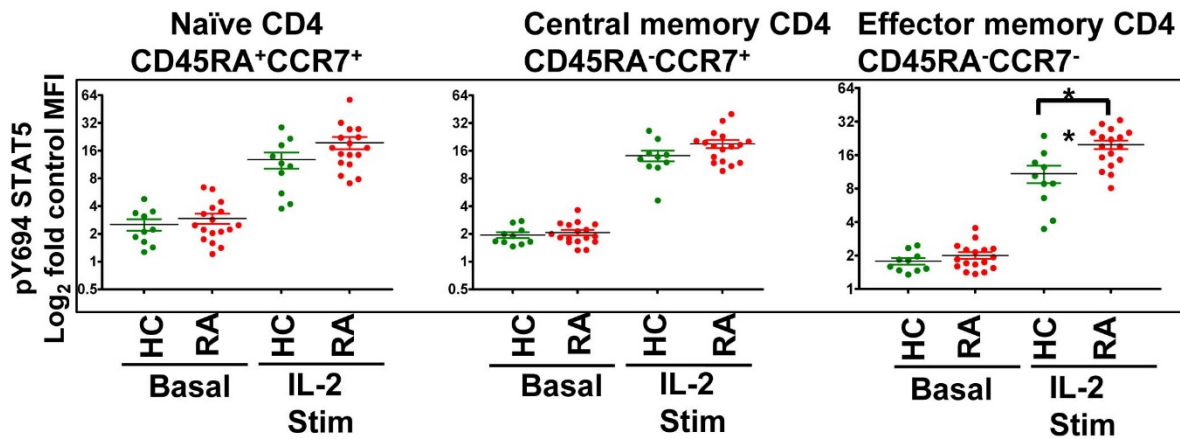

Fig. 2. IL-2 induced activation of STAT5 in CD4 T cells subpopulations from RA and HC. Three populations of CD4 T cells are shown from RA (n=17) and HC (n=10) for levels of pSTAT5 at basal and following stimulation with IL-2 (10 ng/ml) for 15 mins. Basal pSTAT5 was equivalent between RA and HC. IL-2 induced pSTAT5 was significantly higher in effector memory CD4 T cells from RA. *= p<0.05
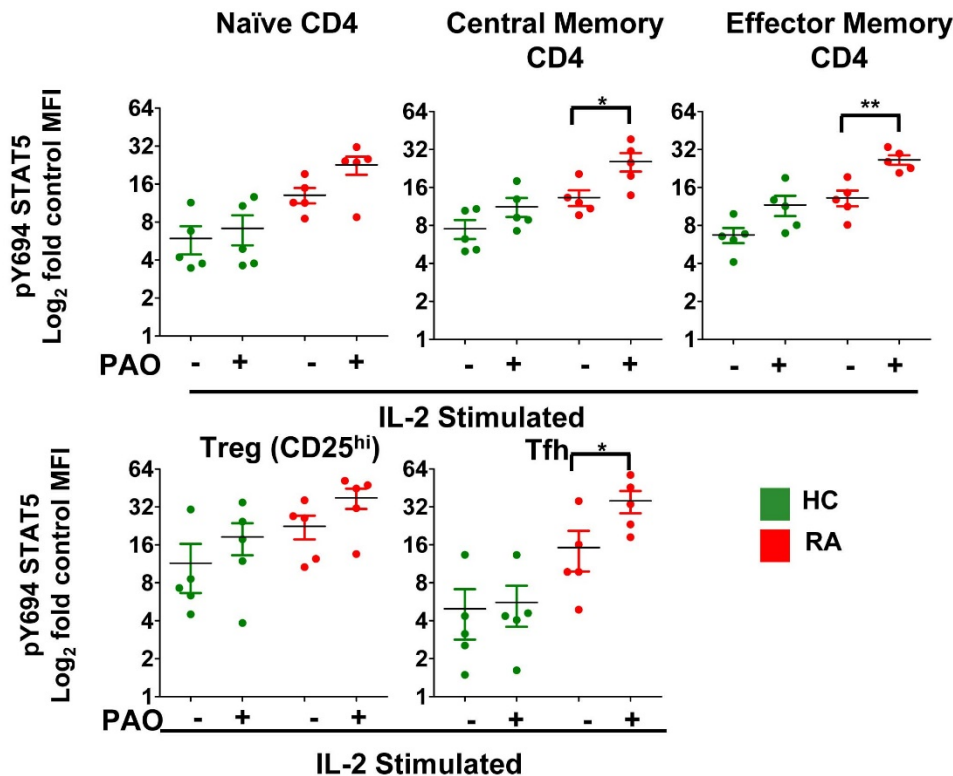

Fig. 3. A pSTAT5 phosphatase attenuates IL-2 induced activation of STAT5 in CD4 T cell populations. CD4 T cell populations from RA (n=5) or HC (n=5) were stimulated with IL-2 in the presence or absence of (PAO), a phosphatase inhibitor). PAO treatment enhanced IL-2 induced STAT5 activation in all CD4 T cell populations, but the difference was significant only for central and effector memory RA T cells. *= p<0.05.

These results are intriguing, and will be pursued further in the next budget period.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

While this project was not intended to provide training and professional development opportunities, it has been an excellent training vehicle for several learners. This project provided opportunities for training for two PhD students, Vishal Sharma, and Brandon Pope, who each joined the Bridges/Raman group in March 2017 and have each developed significant expertise in techniques required for this project and are generating high quality data. Mr. Sharma is a PhD student in the UAB Immunology theme of the UAB Graduate Biomedical Sciences program, and Mr. Pope is an MD/PhD student in the UAB Medical Scientist Training Program (MD/PhD training program). In addition, an undergraduate summer student, Claudia Rose Keating has participated for the past 2 months. All three of these trainees have benefited from mentorship from Drs. Raman and Bridges.

Mr. Sharma and Mr. Pope have been integral parts of the training and educational activities (seminars, lectures, workshops, etc.) of the UAB Division of Clinical Immunology and Rheumatology, and the UAB Comprehensive Arthritis, Musculoskeletal, Bone, and Autoimmunity Center (CAMBAC), one of ~20 university-wide interdisciplinary research centers at UAB. In addition, Mr. Sharma and Pope presented their initial studies at the Southeastern Immunology Conference, June 17-18 (Vanderbilt, Tennessee) and both have submitted abstracts for presentation at the national American College of Rheumatology meeting in November, 2017. Mr. Pope has been nominated for a Student Achievement Award for travel to this meeting.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Goals
1. We anticipate meeting the target for patient recruitment, i.e. 150 RA, 50 MS and 50 HC.
2. Aim 1 studies will be mostly completed. This represents two objectives (i) expression of IFNGR1 and IFNGR2 in unstimulated T cell and B cell populations and monocytes and (ii) expression of IFNGR1 and IFNGR2 in CD4 T cells differentiated to Th1, Th17 and Th17/1 (expresses both IFN-γ and IL-17).
3. Complete studies proposed in Aim 2, subtask 1. Currently we analyze 8 samples per week, leaving enough time for data analysis. For data analysis will use a combination of standard flow cytometric analysis using FlowJo combined with t-SNE. This approach offers us the opportunity to analyze the data to determine activation changes in subpopulations of T cells that are present in low frequency.
4. In a subset of patients, we will utilize imaging flow cytometry (ImageStream) to quantitate translocation of activated STATs into the nucleus (objective of Aim 2 – subtask 2).
5. We will interrogate if activation of T cells by other cytokines, such as IL-2, GM-CSF, IL-6 etc) are affected by IFN-γ stimulation.
6. Experiments to determine outcome of IL-2 induced activation of STAT5 in RA and controls will performed along with IFN-γ stimulation. We therefore expect to analyze majority of the patient samples by end of year 2.
7. Data analysis with the statistical help of Dr. Reynolds (Co-Investigator) will be performed continuously during year 2. We expect to submit a manuscript for publication during year 2.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

There were no significant changes to the approach during this reporting period.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

There were no significant problems or delays encountered during this reporting period.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

There were no changes during the reporting period that had a significant impact on expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No significant changes.

Significant changes in use or care of vertebrate animals.

No significant changes.

Significant changes in use of biohazards and/or select agents

No significant changes.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*

- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	*S. Louis Bridges, Jr., MD, PhD*
Project Role:	*Principal Investigator*
Researcher Identifier:	*ORCID ID: 0000-0003-3785-1389*
Nearest person month worked:	*1.2 calendar months*
Contribution to Project:	*Dr. Bridges has provided overall guidance for this project. He leads the effort to identify patients to be enrolled, oversees all studies in Aim 1 and works closely with Dr. Raman on all lab-based studies in the project. He supervises the Laboratory Manager (Mr. Wanzeck) and all non-lab based study personnel. He oversees the collection of clinical data, processing of blood samples, and all data management aspects of the project.*

Name:	*Chander Raman, PhD*
Project Role:	*Co-Investigator*
Nearest person month worked:	*4.8 calendar months*
Contribution to Project:	*Dr. Raman is critical to the success of this project. He directs and oversee the functional/mechanistic studies. He directly oversees all lab-based research personnel except for Mr. Wanzeck. Dr. Raman works closely with the PI on all three Aims of this project and is key to data analysis, manuscript preparation and submission.*

Name:	*Richard Reynolds, PhD*
Project Role:	*Co-Investigator*
Nearest person month worked:	*1.2 calendar months*
Contribution to Project:	*Dr. Reynolds has provided direct into the study design, overall analysis plan, and statistical analyses for the project.*

Name:	*Keith Wanzeck, BS*

Project Role:	Laboratory Manager
Nearest person month worked:	1.2 calendar months
Contribution to Project:	Mr. Wanzeck is be responsible for coordination of blood draws, and processing and routing of blood samples and biospecimens. He serves as a liaison between Dr. Bridges' lab and Dr. Raman's lab.

Name:	Stephanie Ledbetter, MS
Project Role:	Program Manager
Nearest person month worked:	1.2 calendar months
Contribution to Project:	Ms. Ledbetter is responsible for all regulatory issues, including the UAB IRB submissions and renewals, as well as HRPO issues. She also coordinates other aspects of the study such as laboratory meetings, and other logistic issues.

Name:	Jinyi Wang
Project Role:	Research Associate
Nearest person month worked:	12 calendar months
Contribution to Project:	Ms. Wang is responsible for performing isolation of PBMCs, and plays a large role in all the experiments done in all aims. She helps to guide and supervise the graduate students and other trainees in the lab-based research procedures performed as part of this study.

Name:	Vishal Sharma
Project Role:	Graduate student
Nearest person month worked:	4 calendar months
Contribution to Project:	As part of his PhD studies, Mr. Sharma is performing dissertation research on this project. He works on performing the assays, data analysis, and presentation of results from this project, and beginning to plan follow up studies.

Name:	Brandon Pope
Project Role:	MD/PhD student
Nearest person month worked:	4 calendar months
Contribution to Project:	As part of his MD/PhD program, Mr. Pope is performing dissertation research on this project. HE works on performing the assays, data analysis, and presentation of results from this project, and beginning to plan follow up studies.
Funding Support:	Medical Scientist Training Program grant

Name:	Claudia Rose Keating

Project Role:	*Summer Undergraduate Student*
Researcher Identifier:	*N.A.*
Nearest person month worked:	*2 calendar months*
Contribution to Project:	*During the summer, Ms. Keating has worked on Aim 2, subtask 1 experiments and data analysis.*
Funding Support:	*Institutional funds*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

See attached Other Support documents for Drs. Bridges, Raman, and Reynolds.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:

<u>*Organization Name:*</u>
<u>*Location of Organization:*</u> *(if foreign location list country)*
<u>*Partner's contribution to the project*</u> *(identify one or more)*
- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

Not applicable.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Not applicable.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None.